preparing 20 mM Hepes, pH 7.9, 400 mM NaCl, 0.2 mM EDTA, 20% glycerol, 1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, 1 μg/ml antipain , 1 μg/ml leupeptin.

8. Newly submitted) The method of claim 2 wherein the step of preparing cytoplasmic extraction clarification buffer identified as buffer D comprises:

preparing 20 mM Hepes, pH 7.9, 400 mM NaCl, 0.2 mM EDTA, 40% glycerol, 1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, 1 μg/ml antipain , 1 μg/ml leupeptin.

REMARKS

Recapitulation of the claim for the convenience of the Examiner set forth below is a recapitulation of the claims in the present application.

Claim	Status	Dependency
1	Cancelled	-
2	Newly submitted	Independent
3	Newly submitted	2
4	Newly submitted	2
5	Newly submitted	2
6	Newly submitted	2
7	Newly submitted	2
8	Newly submitted	2

The comments of Examiner Dr. Whisenant have been reviewed carefully. The

application has been amended in accordance with Examiner Dr. Whisenant's requirements and favorable reconsideration is solicited earnestly.

The status of the Claims are as follows: Claim 1 has been cancelled. The subject matter of Claim 1 has been rewritten to eliminate prolixity and to remedy the informalities which has been correctly noted by the Examiner. The subject matter of Claim 1 is presented in newly submitted Claims 2-8.

Claims 2-8 do not include any new subject matter.

It is noted that the Examiner previously found Claim 1 to be allowable.

The Examiner has noted a problem in opening the disc containing the sequence information submitted with the previous amendment. A copy of the disc previously submitted with the amendment was examined by the Applicant was found to be satisfactory. A new disc has been prepared and is submitted with this Amendment.

It is noted that Claims 2-8 (newly submitted) do not claim any particular sequence motif since any relevant DNA sequence can be used for application of the method of present invention.

With regard to the Examiner's request concerning a copy of Dr. Caruccio's dissertation or a paper from the Applicant declaring that the claimed method of the present Application is not disclosed in this dissertation. The Applicant has prepared a paper, attached herewith, stating that

the claimed method of the present Application is not disclosed in Dr. Caruccio's dissertation.

The Examiner has note that Das et al., 1990, teach simultaneous isolation of DNA, RNA and protein . Examination of the method as presentation by Das et al., indicates that the method of Das et al., differs from the present invention as follows:

a) The Applicant has observed in laboratory tests that the use of Ficoll, Percoll, beta-marcapthoethanol, sucrose and spermine, as disclosed by Das et al., distorts the sequence specific DNA-binding protein isolation from the mammalian cells. The method of Das et al., does not provide useful or reliable results for mammalian cells

b) The Applicant has also observed in laboratory tests that the success of pestle grinding method as described by Das et al.,depends on the individual researcher's technique and skill, and the cell types to release cellular protein from the cells. This variability has been noted by Das et al., on page 214, item 3 of their paper. The Applicant has used the pestle grind method dated back to 1987 for his initial publications and has subsequently omitted this grinding method in the present application, thereby, eliminating the problems of variability and lack of repeatability. Moreover, this invention successfully increases the yield of protein and DNA.

c) Most importantly, the method described in the current Application significantly reduces the repeated handling of the biological and human materials, which is inherent in the method of Das et al., and, thereby, reduces the contact of the researchers with the blood-borne and other disease causing pathogens.

d) The method of Das et al., does not teach the yield of intact transcriptionally active protein for the DNA-protein interaction assays or the intactness of high molecular DNA greater than 32kb as revealed from their Southern blot data. Moreover, in vitro run-on transcription assay using the isolated nuclei and gel data as proposed by Das et al., does not really substantiate the presence of intact and undegraded biologically active proteins with the proper motifs and conformation for sequence binding, as observed by the Applicant in the laboratory.

e) The method as proposed by Das et al., using frozen tissues, processed with chloroform, has been found not to be suitable for optimal DNA-binding protein isolation by the Applicant in the laboratory, since it increases the degradation of protein and inefficient DNA-protein interaction.

f) The frequent distortion and variability in yield of protein and DNA from batch to bath in the method of Das et al., has been eliminated using the

method of the present Application. For the reasons cited above the publication of Das et al., reference is not considered appropriate. There is no teaching in Das et al., which leads to the usefulness of the method of present Application.

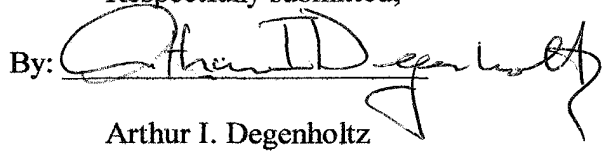
The Examiner has previously stated that Claim 1 is directed to allowable subject matter. Claims 2-8 eliminate the prolixity of Claims and are believed to patentable.

Applicant respectfully requests that a timely Notice of Allowance be issued in this Application.

A petition and petition fee covering a one month extension in the time to respond to the Office Action dated December 20, 2001 are attached herewith.

Courtesy, cooperation and skill of the Examiner Dr. Ethan Whisenant are acknowledged.

Respectfully submitted,

By: 

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Dated: April 17, 2002

Attachments:

Statement of Applicant,

Petition for extension of time

A check in the amount of $55.00

Sequence on a disk.